DESIGN OF A VERSATILE AND DISPOSABLE MICROFLUIDIC CHIP FOR AUTOMATED SAMPLE PREPARATION AND NUCLEIC ACID EXTRACTION

by

John Glenn Minson

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STATEMENT OF THESIS APPROVAL

The following faculty members served as the supervisory committee chair and members for the thesis of **John Glenn Minson**.

Dates at right indicate the members' approval of the thesis.

Bruce Gale, Chair	**May 7, 2013** Date Approved
Tim Ameel, Member	**May 7, 2013** Date Approved
A. K. Balaji, Member	**May 7, 2013** Date Approved

The thesis has also been approved by **Tim Ameel**

Chair of the Department/School/College of **Mechanical Engineering**

and by Donna M. White, Interim Dean of The Graduate School.

ABSTRACT

Significant progress has been made in recent years advancing microfluidic extraction systems for nucleic acids (NAs). However, much remains to be done in the area of sample preparation as it presents significant challenges and additional steps before nucleic acids can be extracted. These difficulties prompted the design of a versatile, disposable extraction system that was robust enough to handle a wide range of raw biological samples and perform the necessary sample preparation protocols. This design was accomplished through an iterative design process, building on Johnsons's polydimethylsiloxane (PDMS) extraction chip [1]. With a primary focus on reducing cost and improving manufacturing time, a new chip was designed and constructed from 3 layers of laminated polycarbonate and a silicone flexible membrane layer. Four features were adapted or redesigned to make the chip fully function: the microvalves, fluid inlet connections, reservoir-pumps and microfilter. Significant design changes were used to make this integration possible. The most notable change involved removing the lower portion of Johnson’s PDMS chip[1] and integrating it as a permanent fixture on the pneumatic actuation system. This modification greatly simplified the chip, minimizing cost and manufacturing time while allowing the microvalves and reservoir-pumps to function exactly as before. In addition to designing a new extraction chip, the supporting pneumatic actuation system was

redesigned and rebuilt as well. Pneumatic failures in Johnson's pneumatic machine were common, which were caused by an excessive amount of flexible tubing and connectors. To create a more reliable pneumatic machine, a central manifold was constructed with access to the necessary pressure sources. The new system was tested with water for basic fluidic functionality. It successfully demonstrated working valves, reservoir pumps and filter flow. Subsequent testing revealed the successful extraction of DNA from a purified sample. The new extraction system is simpler, easier to use and fabricated in 1/16th of the time and produced for 1/60th of the cost of Johnson's PDMS chip.

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CHAPTER 1

INTRODUCTION

Nucleic acids (NAs) are the central players in all of biology and play a vital role in the fields of biotechnology and medicine [2]. NAs are often the main analysis target because of their ubiquity, stability and specificity [1]. NAs are the focus of a wide range of applications vital to modern medicine and research, including things like: DNA sequencing, biological antigen detection, high throughput genotyping [3], disease pathogenesis, retro virus detection, forensics [4], and infectious disease identification [5].

Traditional clinical diagnostic and biological detection analyses typically occur in centralized labs where extensive sample manipulation, manual handling and large laboratory equipment are the norm [4]. These skill dependent operations must be conducted in labs with appropriate bio-safety classifications limiting the overall availability of DNA/RNA analysis [5]. This combination of factors leads to limited portability, increased cost and long analysis times [4]. These limitations are especially problematic in medical diagnostics when whole populations need to be tested [6, 7].

Microfluidic systems have started addressing these issues in recent years. Significant effort has been invested in developing microfluidic “lab-on-a-chip” devices that can extract NAs for a range of applications. Microfluidic systems have demonstrated a number of advantages including: ease of operation, increased detection sensitivity, low cost, faster results and greater mobility [8, 9].

Extraction of NAs is a multiple step process. The sample must first be ruptured or lysed to release the NAs and molecules from within the cells. Lysing can be done by a number of methods including mechanical, chemical, thermal, and electrical lysing [9]. The NAs must then be extracted or purified from the other molecules. The typical method used to extract NAs in microchips is solid phase extraction (SPE). SPE can be accomplished in a variety of ways including silica-based surface affinity [8, 10], electrostatic interaction [11], nanoporous membrane filtration [12, 13] and functionalized microparticles [9, 14].

While many devices have successfully extracted NAs from a prepared sample, relatively few devices can extract NAs from raw biological samples because of the multiple steps involved [9, 15]. Sample preparation protocols are highly specific operations increasing the complexity of the microfluidic chip, often leading to specialized chip designs that perform a single specific task [16]. In recent years, a number of devices have been produced that can extract NAs from raw samples. These new devices have can be grouped based on the described innovation or focus of the device: extraction efficiency [11, 17-26], new materials [27, 28], novel methods [7, 29-34], extraction time [14, 35, 36] or automated processes [37-41]. While all of these objectives are valid, a significant

practical impediment to realizing true “lab-on-a-chip” devices remains the lack of a highly cost effective and versatile solution. Thus, developing a microfluidic chip that is cheap enough to be considered disposable, but robust enough to handle a wide variety of tests and procedures would be a significant contribution.

Many research teams have worked to accomplish a complete lab-on-a-chip system with integrated DNA extraction. Several examples are available in the literature. As an early example, a nominally “disposable” raw sample DNA purification device was developed by Kim et al. [2] in 2002. They extracted DNA from a whole blood sample. The device included a nickel microfilter, polydimethylsiloxane (PDMS) micromixer and a photosensitive glass DNA purification chip. In addition to these materials, a number of complex manufacturing processes were used to create the various characteristics of the chip including thick photoresist and electroplating technology, potassium hydroxide (KOH) etching, UV exposure and high temperature heat treatment. While this approach successfully extracted DNA from whole blood, it requires multiple materials, significant time and complex manufacturing processes. These factors make it difficult to consider this a truly disposable solution.

Disposable devices have received more focus in recent years. By 2009 a number of devices were developed. Mahalanabis et al. developed a disposable microfluidic chip that could extract DNA from whole blood samples [42]. The chip was manufactured from cyclic olefin polymer (COP) with hot embossing techniques and features a microscale silica bead/polymer composite SPE column used for both lysing and extraction. The cell lysing is done by pressure

driven flow through the column in parallel with chemical lysing. While the chip design is extremely simple and disposable, it has only a single input and output requiring manual interchange of the syringe for each of the different reagents used. The chip also lacks features like mixers and reservoirs needed to provide the versatility required for different sample types.

Ling et al. developed a fully enclosed, self contained, disposable microfluidic chip for diagnostic applications [5]. The chip is constructed from silicon and glass while the supporting structure is made from PDMS, polypropylene and polycarbonate. Channels, reservoirs and valves were manufactured using “casting or injecting/micromachining”. A waste bag collects and seals all waste fluids and mechanical actuators control fluid flow. This approach involves a number of materials and manufacturing processes. The chip also has a limited ability to perform multiple testing procedures.

A hands-free, sample preparation device made from a disposable polymer was developed by Baier et al. [6]. Likewise, it contains all on-chip reagents and is actuated by two syringe pumps. The chip is made from cyclic olefin copolymer (COC) and solvent bonded with a cyclic olefin polymer (COP) cover. All chip features were fabricated via milling. Three rotating valves (housing and valve body) were injection molded in polyether ether ketone (PEEK). The disposable valves are electrically actuated by the machine. This system remains limited in its ability to perform a wide range of tests. The chip also requires multiple disposable parts.

Sauer-Budge et al. developed a low-cost, disposable microfluidic chip that could perform sample preparation, extraction and amplification of DNA [43]. The planar plastic chip contained reservoirs, mixers and a column of embedded silica particles for SPE. To keep costs low, the chip contains no active features like pumps or valves. Instead, it utilizes the off-chip pumps to drive and control all fluid flow. The machine is connected to the chip with a clamping mechanism and O-rings seal around the inlets. The device can also perform PCR amplification and fluorescent detection. While this is an elegant solution, the fluid is transferred between the machine and disposable chip introducing the potential for cross contamination and necessitating cleaning of the machine for each use. In addition, the process used to fabricate the SPE column is complex and both time and material intensive, increasing chip cost.

In 2010, Mahalanabis et al. [44] designed a disposable sample preparation and DNA amplification device. The chip is made from layers of cyclic olefin polymer (COP) and porous Teflon films. The layers are thermally bonded for 5 minutes at 125ºC. The chip includes a micro SPE column, flap valves, hydrophobic vents and helicase-dependent amplification (HDA) reaction chambers. The valves are passively actuated by internal pressure and the chip is fabricated with HDA chemicals within the mixing chambers. The system was tested and successfully detected small amounts of E-coli and bacterial DNA. The world-to-chip interface is a major drawback in this system. The fluids are driven by a syringe pump through a single inlet. While this simplifies chip design, it

requires the syringe to be changed manually, multiple times for each test. This chip design is also limited to a single particular test.

That same year, a self contained, disposable, sample-to-answer microfluidic cassette was developed by Chen et al. [45]. The cassette houses all necessary components such as reagents, pouches, valves, reaction chambers, membranes and conduit networks. An electronically controlled analyzer provides mechanical actuation for on-chip pouches and valves for flow control. The device also contains vibrating disks for mixing and regulates thermal cycling for post extraction processes. The cassette is made from a 5.84mm thick polycarbonate body with channels and wells CNC machined on its surfaces. A vacuum line is attached to power the fluid flow. This device successfully demonstrated RNA extraction, amplification and detection in a compact and simple form. However, design limitations include fairly complex manufacturing and fabrication methods and the system remains limited to a single type of NA extraction.

Hwang et al. developed a low-cost polymer microfluidic device for bacterial DNA extraction [28]. The purpose of their work was to substitute traditional silica based surface affinity methods of NA extraction with a polymeric alternative. Although ultimately successful in developing a polymeric based chip that worked with SPE, the processes, chemicals and time in manufacturing are substantial. A more practical approach has been to include a silica microfilter into a disposable plastic chip.

In 2011 Govindarajan et al. developed a low-cost, lab-on-a-chip device for use in resource limited areas [15]. The device is constructed from paper, mylar

and repositionable adhesives. The key concept behind the device is that through sequential folding, complex physical and chemical processing steps are accomplished. The design utilizes capillary flows for pumps and manual folding/unfolding for valves. The device can be manufactured in 30 minutes using a laser cutter, but some processes such as loading the buffer storage pad with reagents requires four hours in an elevated temperature vacuum. The concept is a low-cost, disposable, single use device requiring no power source or support machinery and uses minimal chemical reagents. The extraction of the DNA takes about 2 hours and requires a lot of manual handling. Therefore the device would not lend itself well to high volume use.

A robust microfluidic platform was developed by Johnson et al. in 2010 that could extract both DNA and RNA from various raw biological samples [1]. The chip was constructed from two layers of polydimethylsiloxane (PDMS) with a flexible silicone membrane sandwiched in the middle. The chip features 21 fluid inlet/outlets, 42 membrane valves and 10 reservoir-pumps, all on chip. Mixing is accomplished by shuttling fluids back and forth between reservoir-pumps. The chip is controlled by pneumatic actuation valves, programmable software and incorporates a disposable glass microfilter for SPE. Johnson successfully extracted DNA and RNA from E.coli and whole blood. While this system is superior in versatility, it lacks cost effectiveness to make it truly disposable. The glass microfilter is modular and disposable, but the system was designed to be washed and reused due to material cost and manufacture time. PDMS is relatively expensive compared to other materials being used in microfluidic

applications. The chip also takes a number of hours to fabricate from start to finish and fluid connection requires manual handling. A photograph of the extraction chip is shown in Figure 1.

Each of these systems effectively extracted NAs from raw samples. Many were disposable and relatively cost effective, but most were unable to perform more than a single, specific extraction test. While Johnson's PDMS chip demonstrated the most versatility, it remains costly and lacks disposability. There is a real need in microfluidic diagnostics and NA extraction for a programmable, automated, versatile system with extremely low-cost, disposable chips.

Objectives

The purpose of this work was to develop a rugged system that could handle raw biological sample preparation protocols while remaining inexpensive and disposable. A schematic of the extraction system is shown in Figure 2.

The system is defined as the control software, microfluidic chip and the pneumatic machine. The pneumatic machine houses the electronics, pneumatic pumps and off-chip pneumatic actuators. NA extraction is accomplished by connecting the microfluidic chip to the pneumatic machine. The pneumatic pumps supply the necessary pressures and the pneumatic actuators control the microfluidic chip's valves and reservoir-pumps, enabling sample preparation and NA extraction on the chip.

The design and layout of Johnson's PDMS chip [1] served as the starting point for this project as it was the most versatile system, but required some

significant improvement to enable disposable, regular use. Utilizing Johnson's design as a starting point allowed the use of the associated pneumatic machine and software control system already in place. The intent of this work was to cut the cost of the Johnson chip ten fold and to decrease manufacturing time by half. Reducing cost and manufacturing time would enable the system to be truly disposable, ensuring a viable solution to NA sample preparation and extraction procedures. To achieve these goals an extremely inexpensive material and manufacturing methods were needed, which were simple and well suited for both prototyping and mass production. Both materials selection and manufacturing methods will be reviewed as part of this thesis to determine methods for improving the Johnson design.

There is a need for a disposable and versatile microfluidic system capable of handling sample preparation for raw biological samples. While systems have been made with that intent, there remains much room for improvement. This work will build off the Johnson design, utilizing alternative materials and manufacturing methods to reduce cost and minimize manufacturing time, thereby creating a disposable microfluidic chip.

This thesis will detail the design of a universal microfluidic platform with a disposable, composite, planar chip and cover the design processes for the valves, reservoir-pumps, connection ports, integrated silica microfilter and improved pneumatic machine. In this paper, the effectiveness of the new chip will be evaluated, compared and contrasted with Johnson's PDMS chip and offer suggestions for future work.

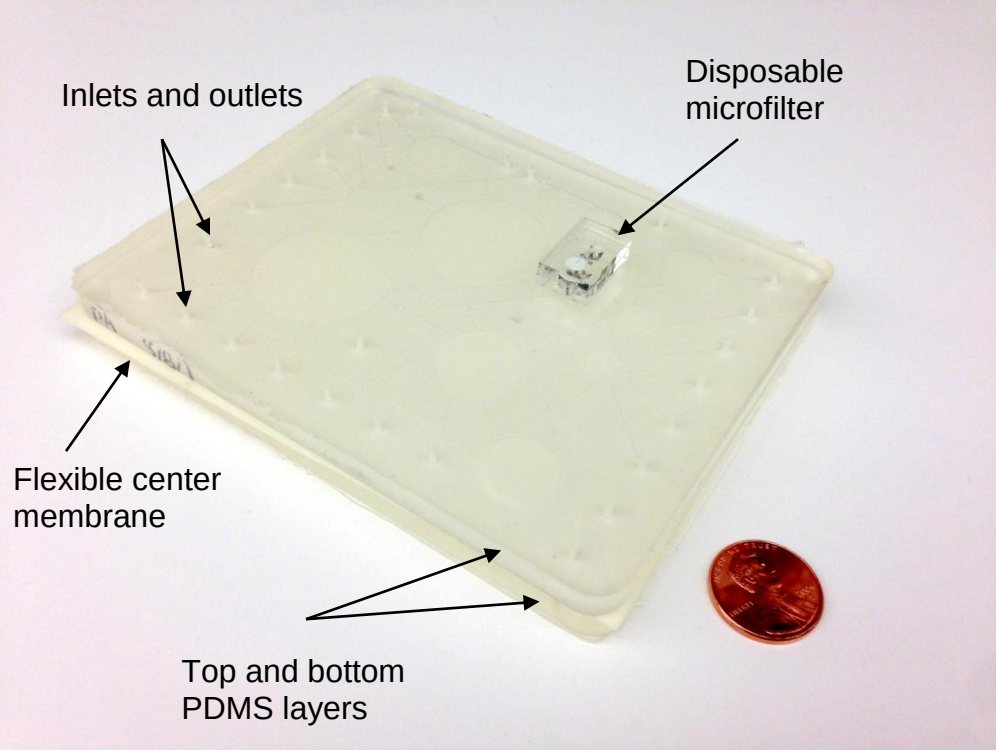

Figure 1: Johnson's three-layered PDMS chip shown with disposable microfilter.

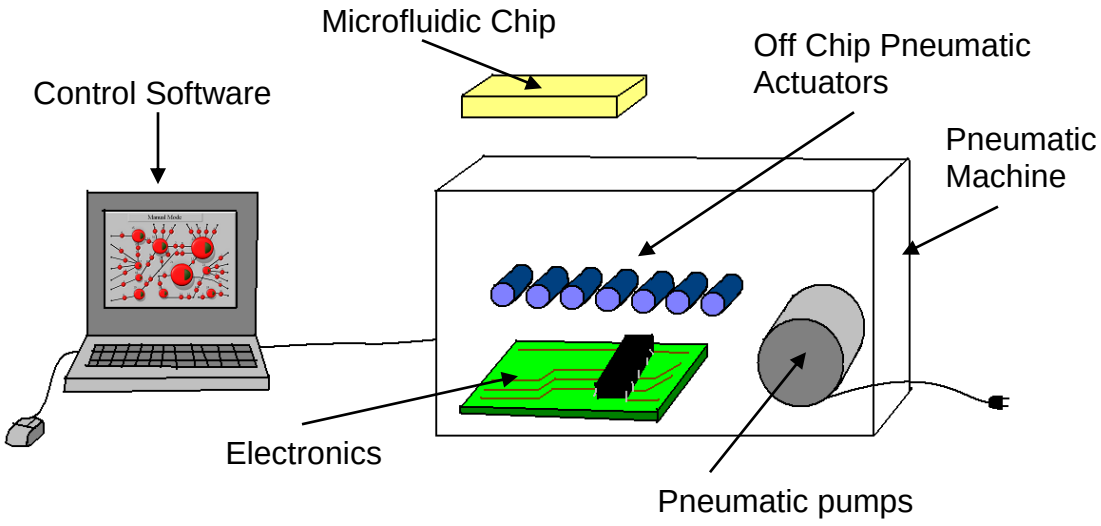

Figure 2: Schematic of the NA extraction system showing the control software, microfluidic chip and the pneumatic machine which houses the electronics, pneumatic off-chip actuators and pneumatic pumps.

CHAPTER 2

DESIGN AND FABRICATION

The popularity of PDMS in microfluidic applications is, in part, due to its versatility. Features like channels, mixers, flexible membranes, valves and reservoirs are relatively easy to make. The material can be made into almost any desirable shape or configuration, but the material is relatively expensive and is not typically used in mass production for a variety of reasons. Careful analysis of the available materials suggested that polycarbonate might be a good choice for an inexpensive yet versatile microfluidic system. While PC costs significantly less and uses rapid and simple methods for manufacturing, it lacks a straightforward approach for similar features because it is thin, comparatively rigid and limited to two-dimensional layering. Developing PC valves, reservoir-pumps and fluid connections required a novel approach.

This approach was discovered while carefully studying Johnson's PDMS chip. On the chip, all fluids pass between the top layer and center membrane. The bottom of the chip is untouched by the fluid and merely serves as supporting structure. Thus, the lower portion could be separated from the chip and integrated as a permanent feature of the pneumatic machine. This concept

enables the PC chip to posses all the same features of the PDMS chip and makes the chip simple, low cost and easy to fabricate while functioning analogous to the PDMS chip.

This new approach required some additional features to the pneumatic machine. A manifold block and clamping lid were designed to seal the PC chip, enabling the use of microvalves, reservoir pumps, fluid connection and glass microfilter. These new additions to the pneumatic machine made sample preparation and NA extraction possible on a PC chip.

During prototype testing of various designs, a number of mechanical issues on Johnson's pneumatic machine disrupted progress. Replacing faulty off-chip pneumatic valves and eliminating pressure leaks became a time intensive task. The hoses used for the pneumatic supplies were connected in series through hundreds of plastic insert connectors and short hose segments. This design resulted in excessive, leaky, tangled hoses. These issues were sufficient motivation to redesign and rebuild the pneumatic machine, incorporating changes that would make it simpler, more reliable and user friendly. The new design was accomplished with a solid, central manifold that housed and distributed the air pressure supply used in the pneumatic machine. This improvement reduced a considerable amount of hoses and connectors and eliminated the pressure leakage issues. The details of this work will be presented in this chapter.

Objectives and Constraints

In order to create a disposable extraction chip, the cost of Johnson's PDMS chip must be reduced significantly. Cost reduction is accomplished by reducing both material cost as well as manufacturing time. The specific objectives of this work include:

- Reduce material cost by a factor of ten
- Decrease manufacturing time by half

These objectives require an exploration of alternative materials that can be manufactured rapidly and inexpensively. In addition to the cost reduction requirements, the materials must also satisfy several constraints:

- Remain biologically compatible with NAs and reagents
- Perform NA extraction in a 30 minute time frame
- Operate within the pressure parameters of the pneumatic machine
- Extract at least 30% DNA from a known concentration

The success of this project hinges on the selection of materials that can satisfy both these objectives and constraints.

Materials

Over the years, a variety of materials have been used in microfluidic applications, with the most dominant being silicon and glass [46]. These well established materials are widely available, but fabrication methods such as photolithography, dry and wet etching, and high temperature bonding [28] are both slow and costly, especially for prototyping and low volume production.

Beginning in the late 1990's, PDMS experienced rapid growth in popularity due to its simple, soft-lithography-based elastomer casting process and comparatively low cost [46]. Other desirable microfluidic traits of PDMS include: optical transparency, flexibility, ease of bonding, impermeable to water, permeable to gas, biologically compatible, and nontoxic to cells [47]. Microvalves, reservoirs and micropumps have been effectively and readily implemented in a variety of designs. However, some drawbacks of PDMS include: hydrophobicity of its surface resulting in nonspecific protein adsorption, incompatibility with high concentrations of some organic solvents, and feature geometry limitations [47]. While some PDMS microfluidic devices have been considered "disposable" [2, 48], cost and manufacturing time are far from optimal, making their adoption as a commercial platform less likely.

Thermoplastics are a promising material for microfluidic devices and have a number of advantages including: negligibly low raw material costs, fabrication costs of molded parts hardly affected by design complexity, proven mass production methods, and a large material class allowing polymer selection for nearly any application. Even parts using high-end materials are suitable for disposable applications [49]. In particular, polycarbonate (PC) is a material being used in microfluidic applications with NAs. PC chips have been used successfully in DNA extraction [50] and polymerase chain reaction (PCR) amplification [51]. This material has the advantage of being extremely inexpensive and can be manufactured with cost effective, simple methods like hot embossing [52] or laminating in a heat press [46].

Accordingly, flame retardant, 5 mil PC sheets (Sabic Polymershapes) were chosen to construct the new, planar, microfluidic chips. This material was selected based upon biological compatibility, availability, low cost and previous working experience from related projects.

PC chips are often cut out in layers on a laser cutter or knife plotter. The layers are aligned and laminated in a heat press for a few minutes. To make fluid connections, a small plastic disk with a central hole is attached to the chip with double-sided tape. Tubing is inserted into the center of the disk and glued in place. PC chips are often used for simple microfluidic applications and generally lack features like reservoirs and valves. The chips are typically constructed with relatively few layers and are commonly less than 1mm thick.

PDMS chips are often made in molds with features that have been cut from tape or plastic using a laser cutter or knife plotter. These parts are fixed to the bottom of a mold to form the reverse features of the chip. PDMS is mixed with a catalyst and degassed to remove bubbles prior to pouring into the mold. The mold cures at an elevated temperature (60ºC) for 4 hours. The PDMS is then removed from the mold and undergoes an air plasma surface treatment to enable bonding to the other layers of the chip. PDMS chips vary in thickness depending on the application, but often remain thick enough (3mm or more) to insert tubing connectors for incoming fluid connection.

In order to use PC with the existing equipment and design, not only would the same features need to be duplicated, but they would need to function within the same footprint and with the same pneumatic system. Using such a different

material in terms of volume and rigidity, with the associated hardware constraints made the transition challenging. The valves, reservoir-pumps, microfilter and fluid interface connections had to be redesigned to work with the PC with as much reliability as Johnson's chip.

Valves

Microvalves are essential to complex microfluidic applications and diverse actuation systems exist for microfluidic valves including: electrical, mechanical, piezoelectric, magnetic, thermal, pneumatic and hydraulic schemes [53]. These actuation systems can be coupled with a variety of valving techniques and materials, offering a vast possibility of options. Some valves used with PC and other stiff materials include paraffin wax valves [54], swollen hydrogel [55], PDMS expandable microsphere composite valves [53], ice valves [56], and monolithic membrane valves [57], to name a few. The criteria was to select or develop a PC microvalve that was compatible with the pneumatic machine's actuation system that was in plane, low profile and reliable, while minimizing materials, machining and assembly time.

The first design concept was a simple valve using only PC. The valve was geometrically similar to the flexible membrane valves used in the PDMS chip. The valve consisted of two channels separated by a thin wall. The top layer of PC was thermally bonded to the channel layer everywhere except directly over the wall. This design allowed pressurized fluid to flex the top layer out of the way as the fluid flowed over the wall into the neighboring channel. The valve is closed

by applying a mechanical force over the top of the wall. The valve is illustrated in Figure 3.

This approach provided a low profile, easy to prototype valve, with no moving parts; however, its high internal resistance required more fluid driving pressure than the pneumatic system could supply. The valve also required additional mechanical actuation to close the valve. While the prototype valve sealed very well, it was incompatible with the pneumatic actuation methodology so other designs were explored. The prototype valve is shown in Figure 4 and Figure 5.

Johnson's PDMS chip utilized a silicone rubber monolithic membrane valve developed by Grover et al. [3]. This design takes advantage of the flexible membrane enabling low pressure actuation. The flexible membrane approach seemed like one of the best methods to utilize the low pressure differential provided by the pneumatic machine and previously displayed successful implementation. Thus, a PC version of the flexible membrane valve was prototyped using the same silicone rubber material from Johnson's chip. Attempts to thermally bond the silicone rubber to the PC were unsuccessful and showed no signs of bonding. Finding a flexible material that would thermally bond to PC was troublesome. A semiflexible urethane layer was tested. Although it bonded in the heat press, it had limited flexibility and a much lower melting temperature. This material selection resulted in valves that were often clogged or that leaked. A synthetic nitrile polymer (acrylonitrile butadiene) material was tested next as it had moderate flexibility and displayed similar polymer

characteristics. The nitrile polymer bonded to the PC and worked significantly better. The material was not as flexible as the silicone rubber, but the design and material combination made a functioning and consistently reliable valve.

After testing thermal bonding techniques for the flexible membrane materials, air plasma surface treatment was attempted as an alternative bonding method. Air plasma surface treatment is commonly used in industrial applications to prepare polymers, metals, glass and textiles for improved surface adhesion for printing, painting, coating, labeling and bonding. Air plasma surface treatment works by blowing air past two high voltage electrodes. This process creates positively charged ion particles which in turn, charge the surfaces the plasma comes in contact with. These positively charged surfaces become more receptive to adhesion. Air plasma surface treatment has been used extensively in bonding PDMS, glass and other common microfluidic materials [58].

Initial attempts to bond the original silicone rubber with the PC chip were moderately successful. The materials showed some bonding, but the bonding was blotchy and nonuniform. Sometimes the bond was weak and other times it was much stronger. The cause of this behavior was unknown, although it may have been influenced by foreign contaminants or atmospheric conditions. Several tests were performed to determine possible influencing parameters. These tests included increasing exposure time to the plasma, varying the distance of the plasma from the treated surfaces, adjusting the amount of pressure applied when bonding materials together after plasma treatment and applying heat to the bond following the plasma treatment. Most of these

parameters revealed little correlation to bond strength; however, when the air plasma surface treatment was followed with thermal bonding techniques, a strong, uniform bond was achieved. Multiple valves were prototyped from the silicone rubber and PC layers and found to operate consistently with the pneumatic machine.

Reservoir-Pumps

Developing a reservoir-pump for a thin PC chip was problematic. The PC chip required eight reservoir-pumps with volumes ranging from 100µl to 1000µl. The thin, rigid PC material did not lend itself well to this type of feature. Initial design prospects included using a separate, disposable, off chip reservoir, increasing the chip footprint or adding additional layers with more channel volume. All of these options increased chip complexity and cost so continued efforts were spent exploring creative alternatives.

After carefully investigating Johnson's chip, important relationships were discovered. Johnson's chip is constructed from three layers; a top layer with channels and fluid inputs, a center, actuation membrane layer and the bottom layer with the reservoir volumes and pneumatics for actuation. This lower layer never comes in contact with the fluids and acts only as an actuation and support structure, as shown in Figure 6.

Separating this lower layer from the chip and incorporating it as a permanent fixture within the pneumatic machine allows the chip to be simple and thin. The chip can then be made with laminated PC channels in the top layers

with a flexible membrane as the bottom layer. This design allows the membrane to be stretched away from the chip and into the cavity of the pneumatic machine when vacuum is applied, creating a reservoir-pump with desired volumes. The membrane returns to the chip when released, remaining planar and low profile, which allows the layered chip to remain extremely thin and inexpensive while functioning identically as Johnson's chip. This new approach is demonstrated in Figure 7.

World-To-Chip Connection

The world-to-chip interfaces for microfluidic systems remains a major challenge in device design [59]. For rigid chips like glass and plastic, the connectors are often manually assembled using adhesives, stickers, clamps, or other bonding techniques. These processes generally require multiple steps, special devices and skilled personnel [60]. For flexible materials like PDMS, press-fit connections are often used because they are relatively easy to incorporate in the device. The connections are made by punching a small hole into the PDMS and inserting the tubing. While these connections are simple and inexpensive, they often require manual insertion. Attempts have been made to design manifold vacuum systems [61], socket-type connections for rigid glass chips [62], adjustable, spring loaded, modular connections [60], heat shrink tubing connections [63] and a variety of other designs. While these attempts strive for easier, compact and more robust methods, they generally increase system complexity and cost.

To make the world-to-chip interface practical and inexpensive, a simple, fast, reversible connection using minimal parts was desired. A gasket mechanism that can seal all of the connections simultaneously seemed to be the best choice. A gasket eliminates additional on-chip parts and connects quickly and simply while being easily reversible for disconnection. Effective gasketing requires a flexible sealing material as well as a clamping or vacuum mechanism for a proper seal. The flexible rubber layer on the bottom side of the new PC chip served as the sealing material for fluid transfer. Both the pneumatic and fluidic interface connections were consolidated to the bottom of the PC chip allowing the clamping lid to be plain, clean and simple. The pneumatic connections remain in the central portion of the manifold block, operating valves and reservoir-pumps while the fluid inlets are lined up along the outside edge for access to reagents and samples. Figure 8 shows the chip sealed within the clamp with reagents positioned along the outside edge.

Microfilter

A glass microfilter is utilized in solid phase extraction (SPE) to separate NAs from undesired molecules. To accomplish this task in the new PC chip, a 4mm, disposable, glass microfiber filter (Whatman) is laminated within the channel layer of the PC chip. Because the microfilter is thicker than the channel layer (0.78mm vs. 0.13mm) large gaps around the edge of the filter remained unbonded using traditional thermal bonding techniques. To eliminate the gap, the chip is placed inside a heat resistant, nonstick, coated paper template with holes

cut out on either side of the filter. This template allows the filter to protrude slightly while tightly sealing the PC up against the filter's edges. This additional step ensures the fluids are forced through the filter enabling proper filtration.

The New PC Chip

The newly designed PC chip used is this work incorporated all of the features and components described so far, many of them in multiple locations on the chip. For example, the new PC chip is designed to support 16 fluid inlet and outlet connections. The PC chip includes 8 reservoir-pumps and 33 valves for fluid manipulation, storage, and mixing. A glass microfilter is incorporated for the extraction of NAs. A single, 35kPa air supply is controlled by a valve and used during the extraction process to dry the filter after NA capture. Microchannels guide the fluids from inlets to reservoir-pumps, through the filter and out to waste. A tab was designed for easy insertion and removal of the PC chip and notches in the side of the PC chip assist with proper alignment in the pneumatic machine. The bottom flexible membrane layer functions to actuate all the fluids via the reservoir-pumps and valves. All features are shown and labeled in Figure 9.

The disposable, composite PC chip is manufactured in four layers and cut out on a 40W laser cutter (Versalaser engraver VLS 3.60). The top three layers are PC and consist of a solid top layer, a central channel layer and a valve/reservoir layer. The bottom sealing layer is made from silicone rubber and has openings for sample and reagent transfer. The four chip layers are displayed in Figure 10. A 4mm diameter glass microfiber filter is sandwiched between the

PC layers with the channel layer. The three PC layers and glass filter are manually aligned and laminated together in a JetPress 12 T-shirt press at 175ºC for 90 seconds.

After the chip is removed from the heat press, the bottom surface is air plasma discharge treated along with the silicone membrane for 30 seconds each. They are laid together and returned to the heat press for another 30 seconds. A piece of non-stick, coated paper is inserted between the PC and silicone membrane, masking only the valves and reservoirs to prevent them from bonding closed. This masking allows the reservoirs and valves to function, but seals the silicone membrane to the polycarbonate along the edges of the chip. The final four layered composite chip is 0.66mm thin and is shown in Figure 11.

The PC chip undergoes a small amount of shrinkage during the thermal bonding process. The chip was measured before and after the shrinkage and found to shrink about 1% in both height and width. The chip was then manufactured 1% larger, allowing it to shrink to the proper size.

The PC chip footprint was minimized by shrinking the diameter of the reservoirs while increasing their depth in the manifold block, thereby maintaining equivalent volumes. In addition, two reservoir-pumps that were not being utilized on the Johnson chip were excluded.

During the prototype manufacturing of the PC chip, the channels occasionally sealed shut near the reservoir openings. The sealing often occurred during elevated temperature or time variations in the heat press. In order to make the PC chip more robust to these problems, the channels were given a 1mm

radius at the reservoir openings. This design feature effectively eliminated the problem.

Manifold Block and Clamp

For the PC chip to work properly, a complete seal must be formed around each of the valves, reservoir pumps and fluid connections, which can be readily accomplished with a clamping mechanism. The mechanism provides even and uniform force over the area of the PC chip, includes adjustability and enables fast clamping and removal. To obtain these features, a unique manifold block and clamp were designed. A 24mm thick aluminum block was machined on a CNC Haas mill to serve as the bottom support structure for the PC chip. Pockets were machined into the block for the reservoir wells and valves. To reduce the force needed to seal the PC chip, a 1mm raised metal gasket was machined around each of the features. The machined manifold block is pictured in Figure 12.

The clamping mechanism is designed to give the chip a full seal with complete adjustability. This is accomplished by hinging the lid from 3/8” brass rods in the lower block. The lid is connected to the rods with #10-32 screws tapped perpendicular through their center. The screws can be turned to adjust the distance of the lid with respect to the block manifold. This design provides a precision fit hinging point, minimizes the number of parts and allows clamping force adjustability for the back corners. Figure 13 shows a side view of the clamping mechanism in open and closed positions.

The front of the lid clamps tightly using two over-center draw latches (Southco) which provide up to 276N each of clamping force. The clamps latch to an aluminum plate fastened to the front of the lid with slotted holes for adjustment to each of the front corners. The plate, latches and lid are shown in Figure 14.

The lid is made from 19mm thick acrylic to provide sufficient stiffness (avoiding sealing issues from material flex) while allowing the user to see the process of extraction, enabling program adjustment as necessary.

Tygon tubes (0.02” Saint-Gobain PPL Corp.) are glued into the side of the manifold block as permanent straws for samples and reagents. An acrylic structure, shown in Figure 15, is attached to each side of the manifold block to hold the vials that contained reagents, samples and waste.

The bottom of the aluminum manifold block has 41 pneumatic connection points. Johnson’s original chip design included 42 on-chip valves controlled by only 14 off-chip pneumatic actuators. As a result, many of the on-chip valves required a common off-chip actuator. In Johnson’s original pneumatic machine, the pneumatic routing was accomplished by branching tubes from a single pneumatic actuation source and connecting to various on-chip valves. This approach led to excessive tubes connectors and pressure leaks. To avoid these problems, a nine layered channel structure was constructed from 1.6mm PC plates and bonded with double sided tape (3M 467) as shown in Figure 16. The new routing system was designed to join the common on-chip valves as well as organize, reduce parts and simplify the connections to the pneumatic machine.

To fully utilize the pneumatic machine for future processes, prototypes or additions, the pneumatic actuation lines are made available for modular use on the manifold block structure, which was accomplished by drilling into the valve lines on the side of the bottom gasket and gluing plastic insert connections for flexible tubing. A photograph of the access ports is shown in Figure 17. The lines remain capped when not in use.

Minson's Pneumatic Machine

Johnson's pneumatic machine developed serious reliability issues as a result of the method used to connect the microfluidic chip to the pneumatic machine. A photograph of Johnson's pneumatic machine is shown in Figure 18.

Johnson's pneumatic machine relies on three different pressure sources to actuate the valves and reservoir-pumps. An external high pressure line (69kPa) is supplied to the pneumatic machine and is used for closing the valves. Two small pneumatic pumps are housed within the pneumatic machine. One pump supplies a low pressure (34kPa) source for closing the reservoir-pumps. The other pump generates a vacuum source (-34kPa) for opening both the valves and reservoir-pumps. The off-chip pneumatic actuators provide a solenoid switch mechanism between a positive and negative pressure. These actuators facilitate the opening and closing of various on-chip features. Fourteen of the actuators operate the valves, switching between open (-34kPa) and closed (69kPa). The remaining actuators operate the reservoir-pumps, switching between open (-34kPa) and closed (34kPa).

The pressure sources on Johnson's pneumatic machine were distributed along flexible tubing that had been spliced dozens of times with T-shaped insert connectors at each actuator. In addition, most off-chip pneumatic actuator outputs branched out to multiple on-chip features increasing the number of hoses and connection points. This configuration resulted in hundreds of potential failure points and air leaks were common and difficult to pinpoint. The inner workings of the system are shown in Figure 19. Working with the pneumatic machine was inefficient and time consuming. Thus, a new approach was conceived to eliminate excessive hoses and improve system reliability.

Instead of relying on flexible tubing as the primary pressure delivery component, a rigid structure is preferred. The structure contains the input pressures and distributes them in an organized and simplified way. This approach reduces the number of hoses needed in the pneumatic machine and eliminates excessive connection points. To this end, a central manifold was constructed. The manifold contains three separate compartments to distribute the incoming pressure sources. An array of tubing connectors are glued along the length of the manifold allowing the off-chip pneumatic actuators to connect to a high and low pressure supply. The manifold is mounted along the center of the pneumatic machine and the actuators are divided into high and low arrays on either side. The new manifold with actuators is shown in Figure 20.

In addition to improving the pneumatic delivery system, Johnson's entire pneumatic machine was disassembled, cleaned up and rebuilt. This included resoldering all electrical connections and creating a new housing structure that

provided greater accessibility to the actuators and electronics. This improved pneumatic machine is distinguished as Minson's pneumatic machine and is pictured in Figure 21.

Significant design changes and novel approaches lead to the successful implementation of a new PC chip. This chip features microvalves, reservoir-pumps, world-to-chip connection and an integrated microfilter to fully realize sample preparation and NA extraction. To make the PC chip possible, a manifold block and clamping mechanism were designed and built to work with the new PC chip and Johnson's pneumatic machine was improved and rebuilt to reduce air leaks.

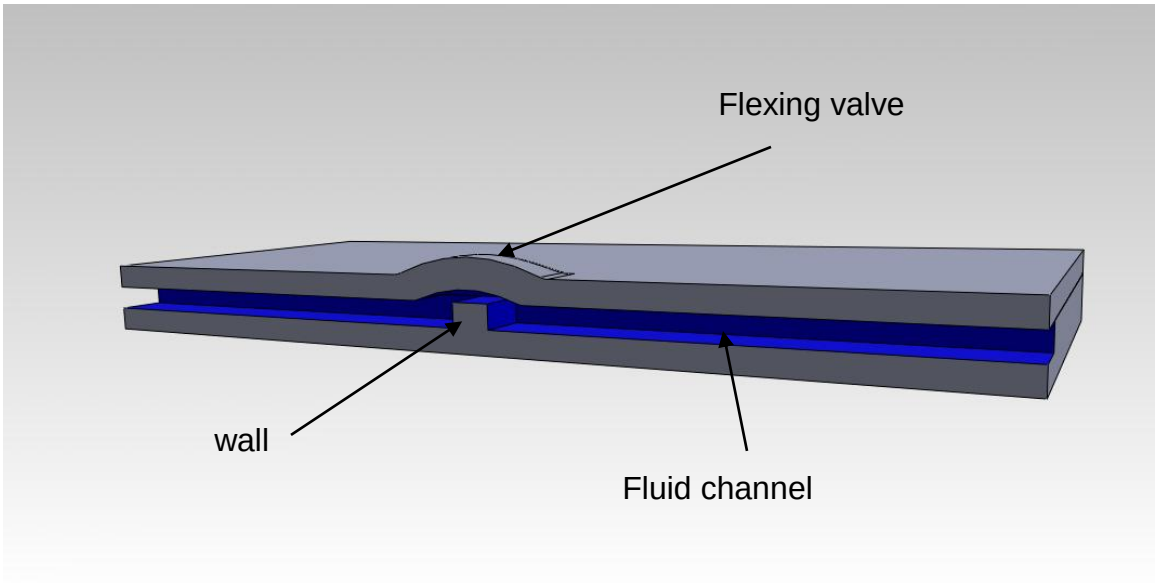

Figure 3: Schematic of first prototype valve, shown in the open position.

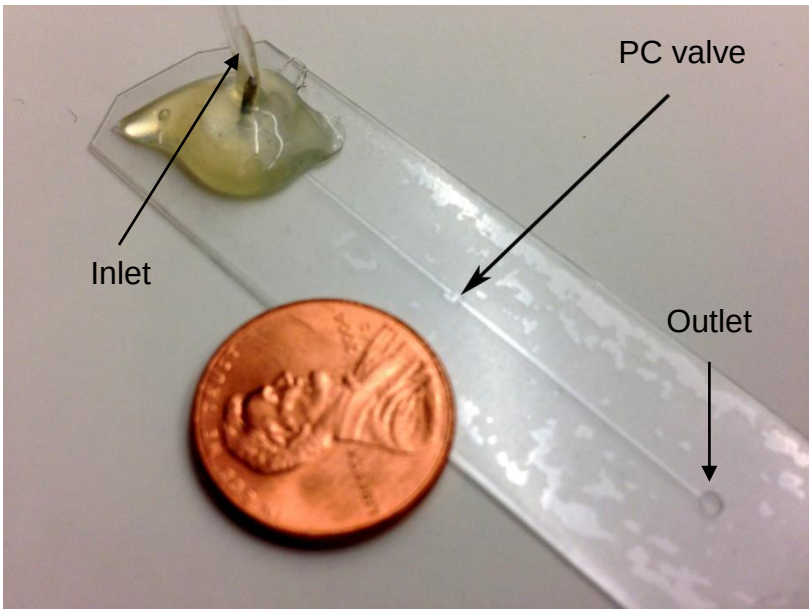

Figure 4: First PC valve prototype shown with fluid inlet connection.

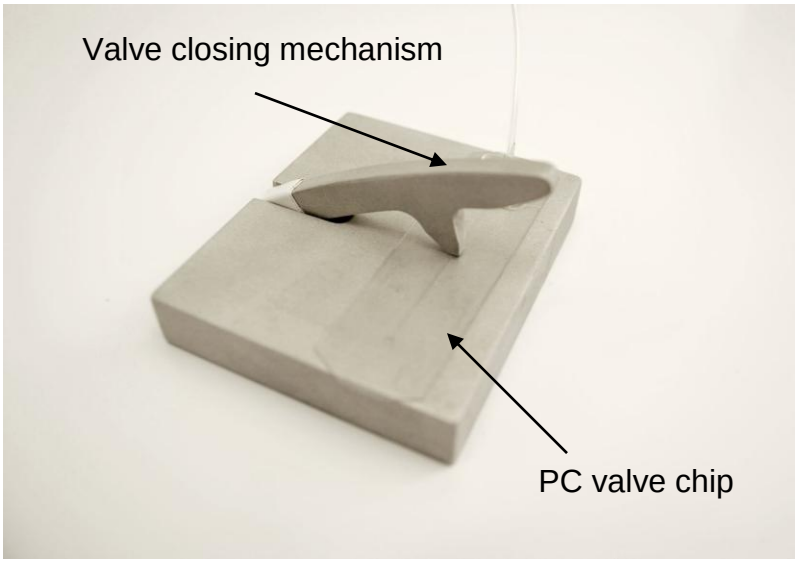

Figure 5: Mechanical actuator prototype with first PC valve chip. A slight downward force on the lever seals valve closed even under very high pressures.

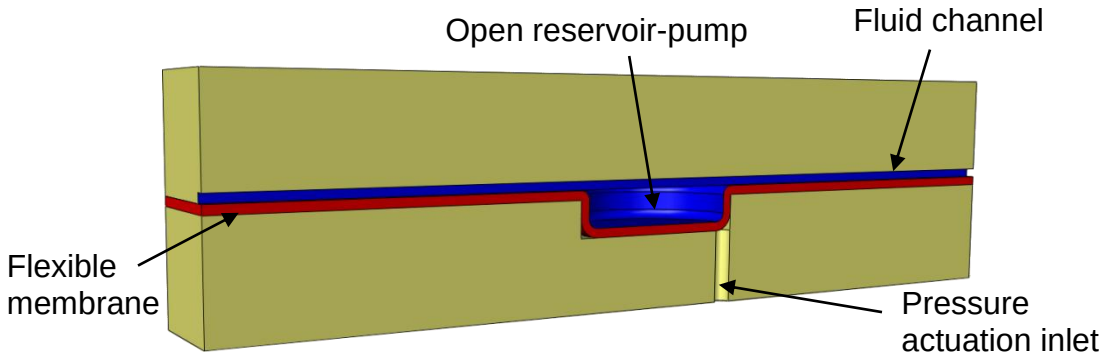

Figure 6: Diagram of the three layered PDMS chip. The blue indicates fluid through the channel and into the reservoir. The red represents the flexible membrane layer separating the upper and lower PDMS layers. The schematic is shown with reservoir-pump in open position.

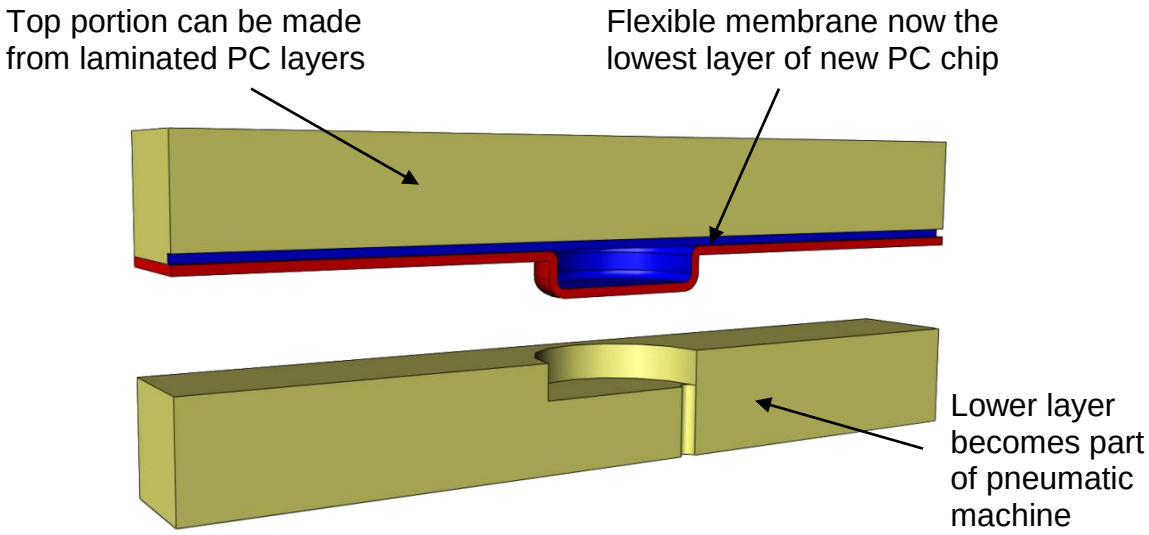

Figure 7: Novel design concept for removing the bottom layer from the chip and including it as a permanent support structure on the pneumatic machine.

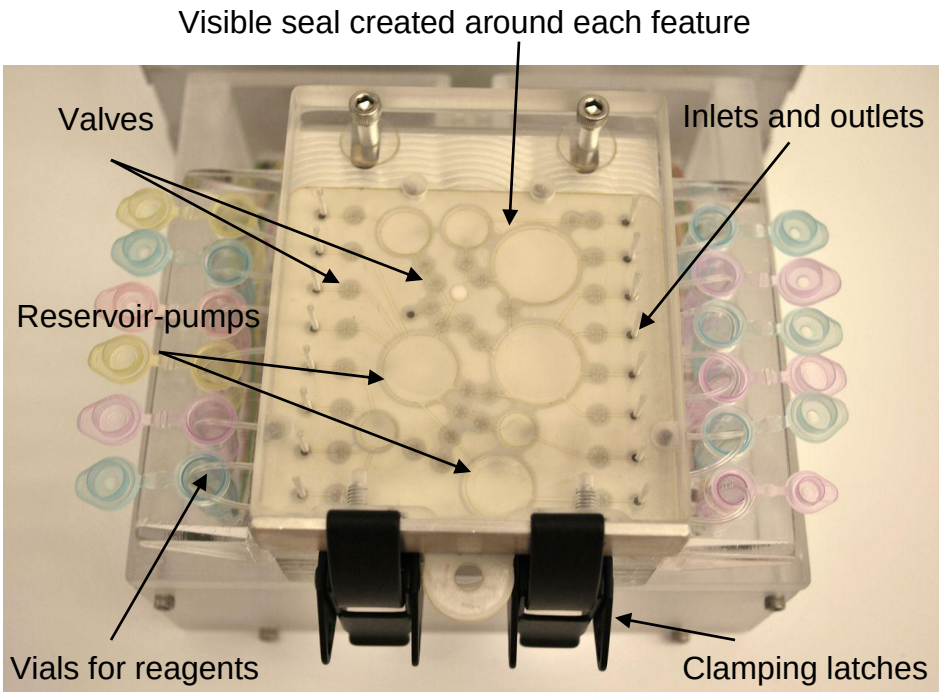

Figure 8: New chip sealed in the pneumatic machine with liquid inlets along outside edge. The seal can be seen as a darker colored ring around the reservoir-pumps and valves.

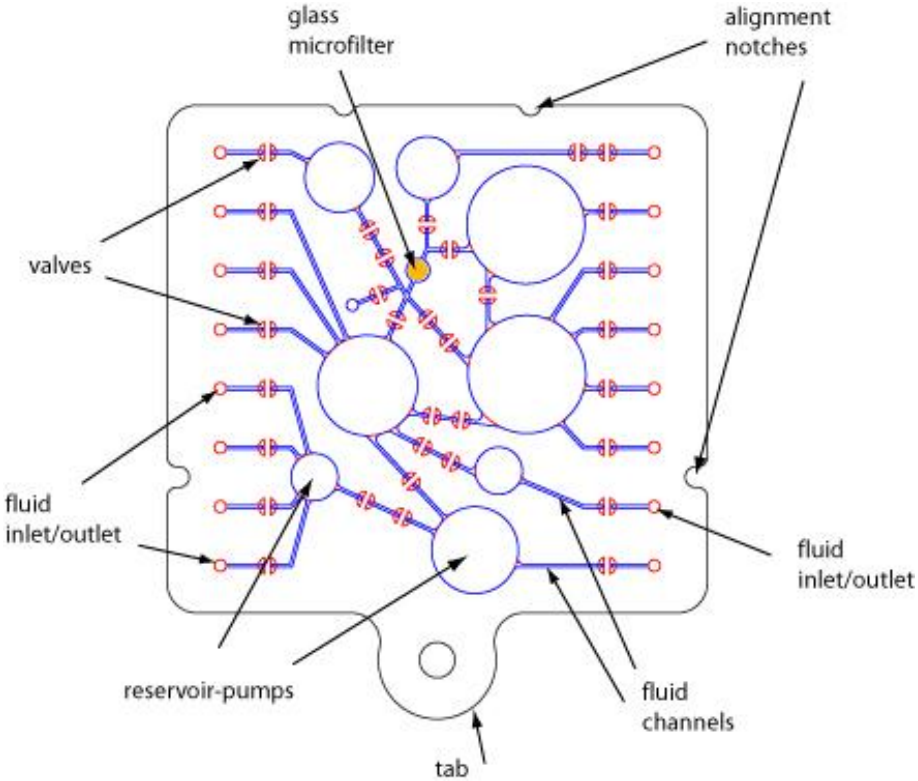

Figure 9: Layout of PC chip with labels indicating the location of various features.

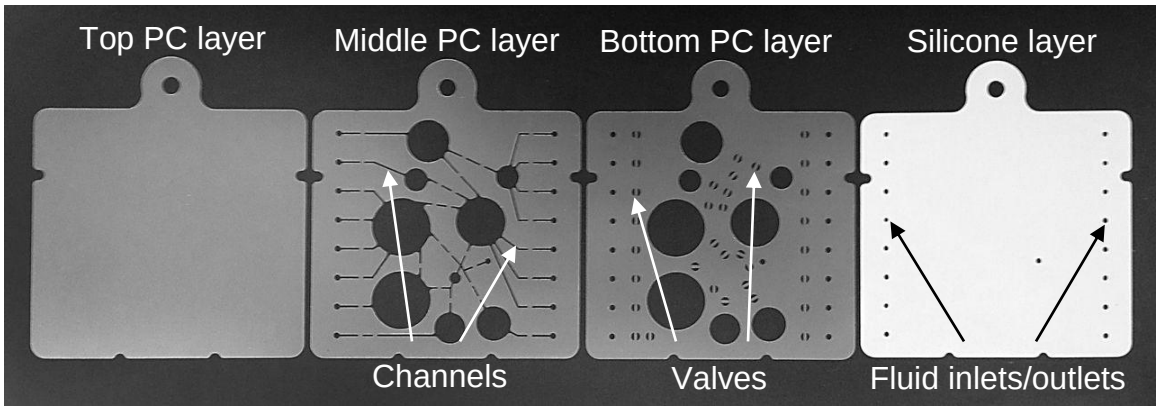

Figure 10: The four layers of the new chip with three PC layers shown on the left and the bottom silicone layer on the right.

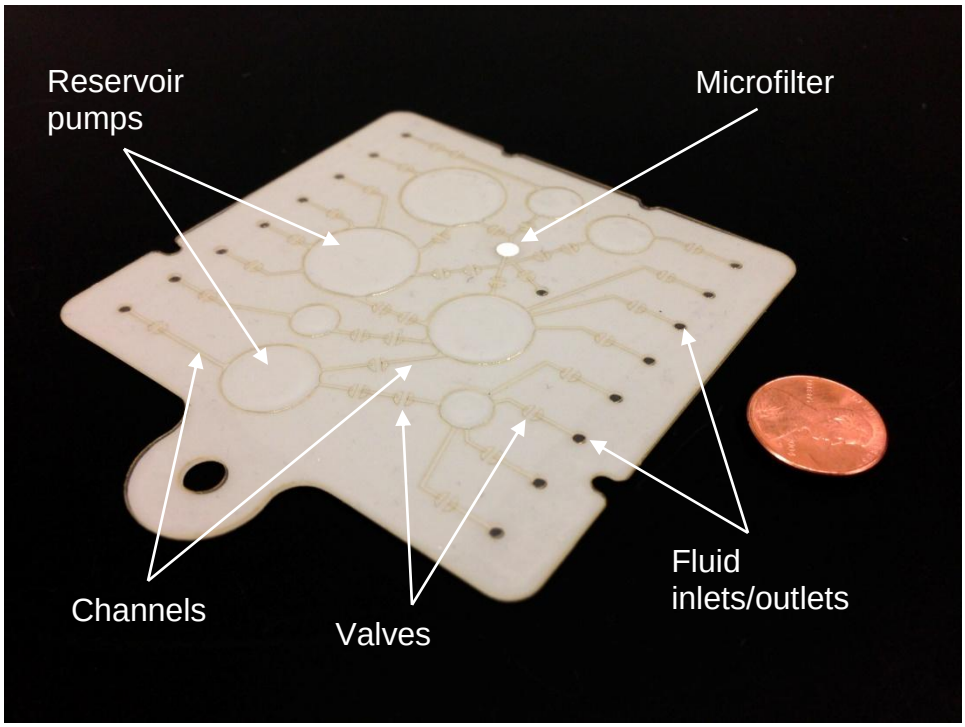

Figure 11: New PC chip fully assembled shown with features.

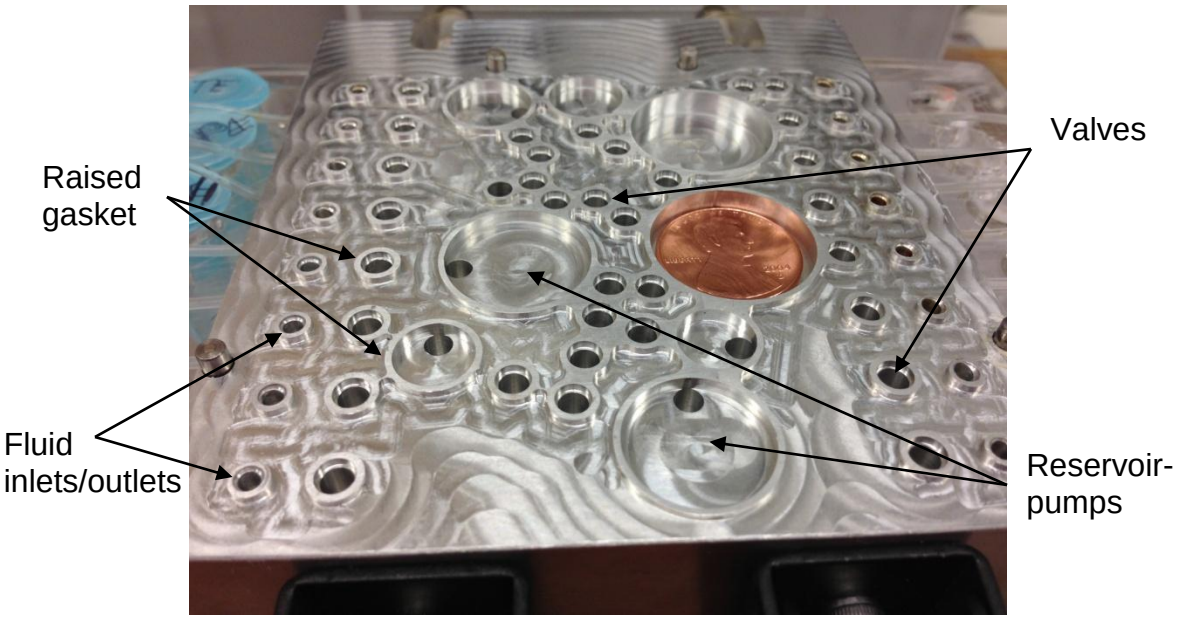

Figure 12: CNC machined manifold aluminum block with a 1mm raised aluminum gasket.

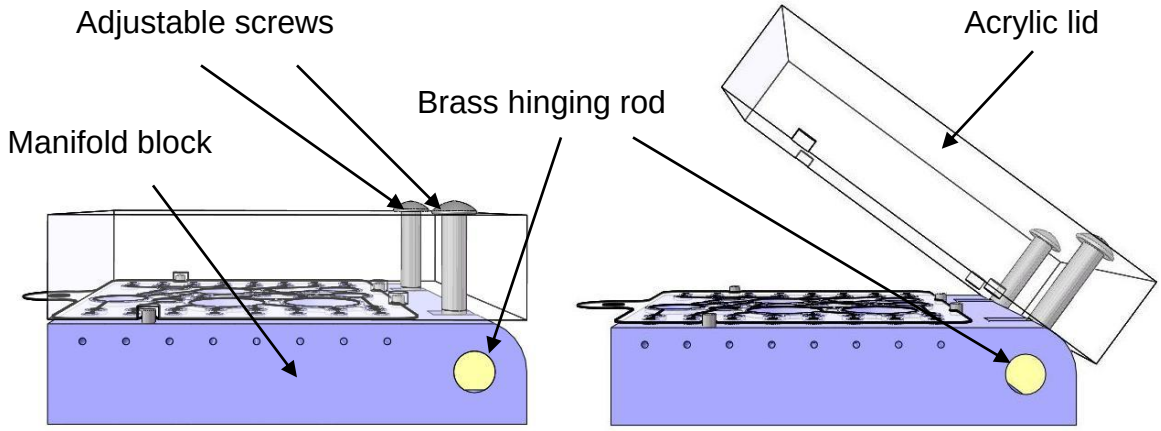

Figure 13: Side view of adjustable clamping mechanism in the closed and open positions.

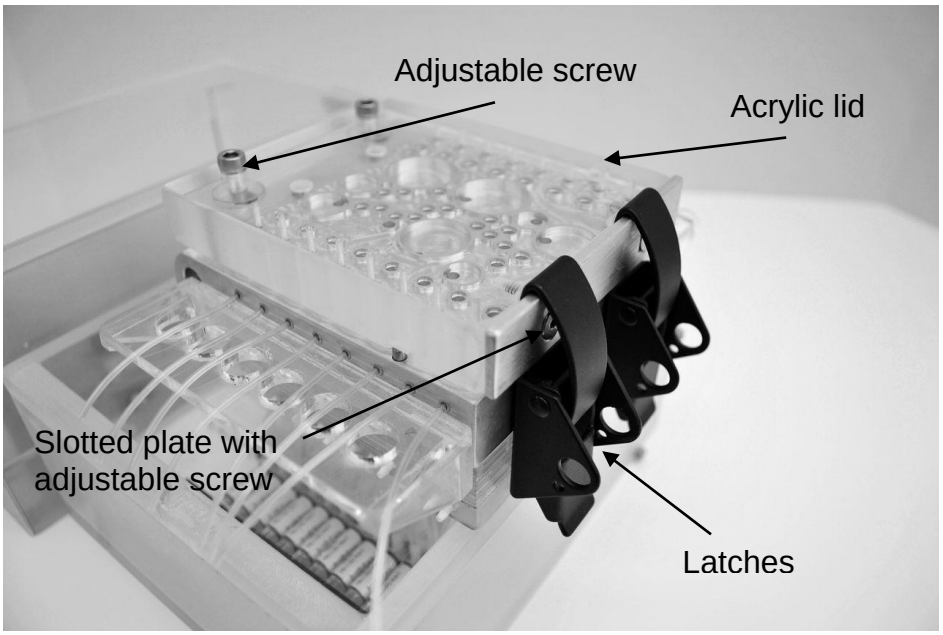

Figure 14: Manifold block and lid with complete clamping adjustability.

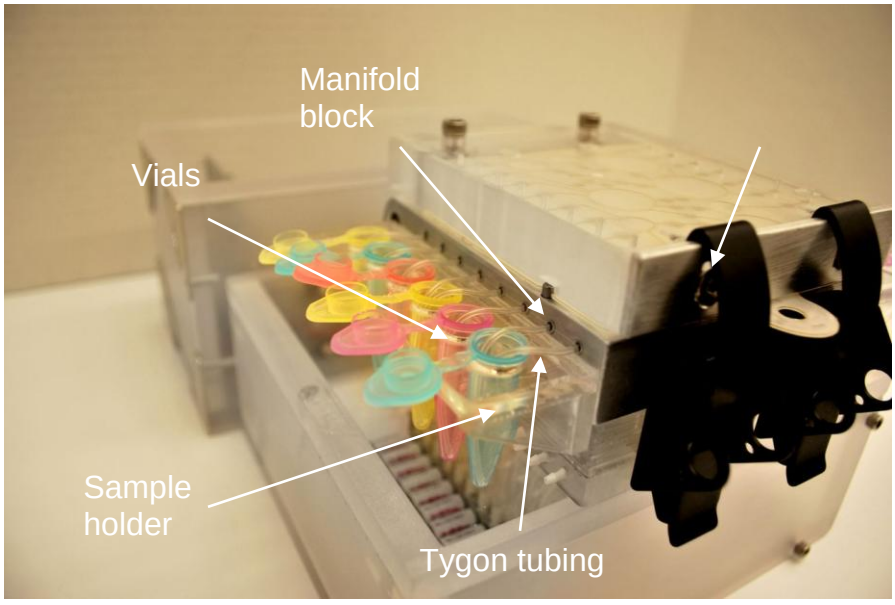

Figure 15: The pneumatic machine with manifold block, acrylic clamping lid, sample holder, vials and tygon straws.

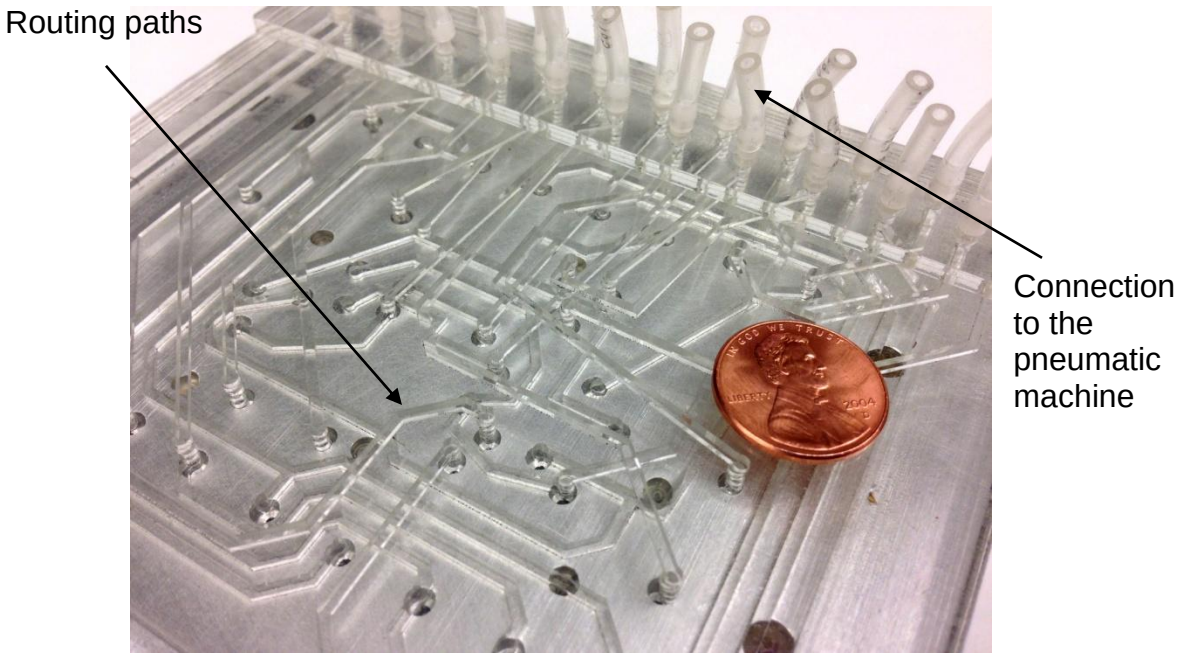

Figure 16: Bottom side of manifold block with nine layered channel organizer to avoid clustered hoses.

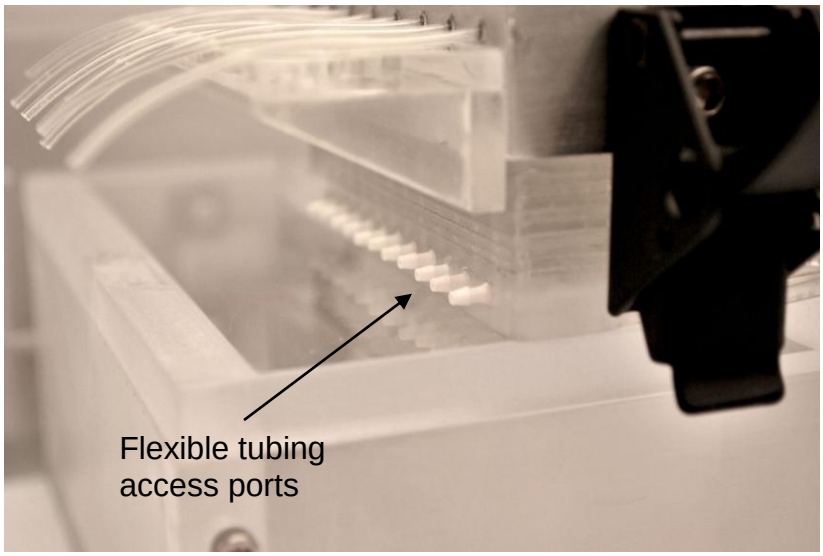

Figure 17: Optional access ports for connecting other prototypes or postprocess procedures utilizing the pneumatic valves and control system.

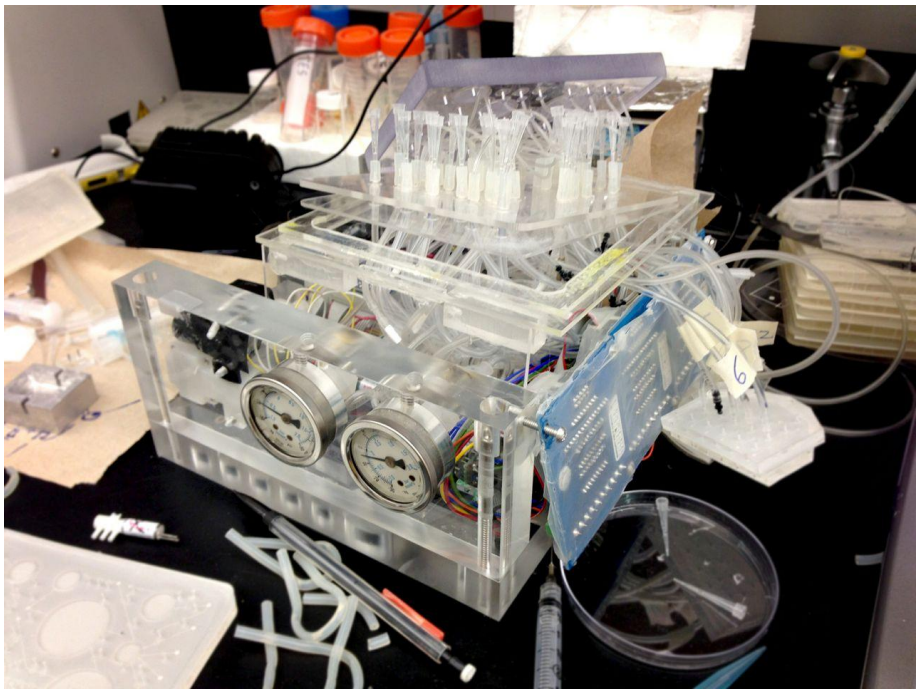

Figure 18: Johnson's pneumatic machine with hose intensive connection points.

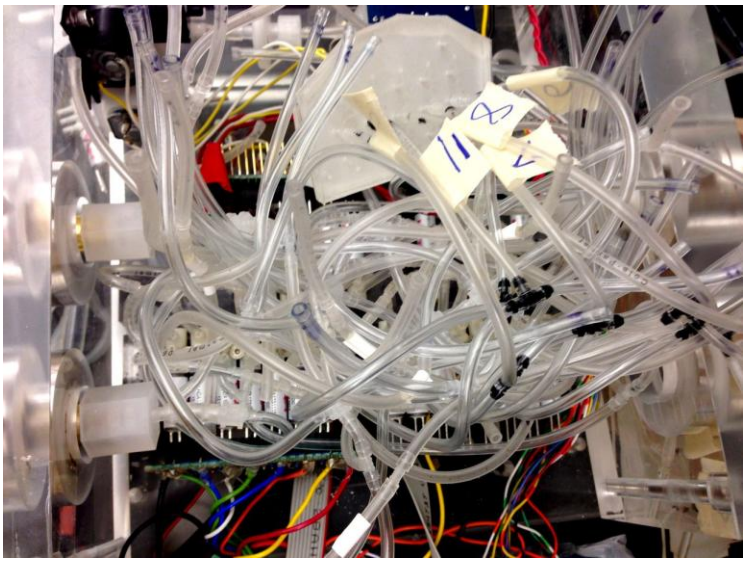

Figure 19: The inner workings of the original pneumatic machine with the hose connection approach.

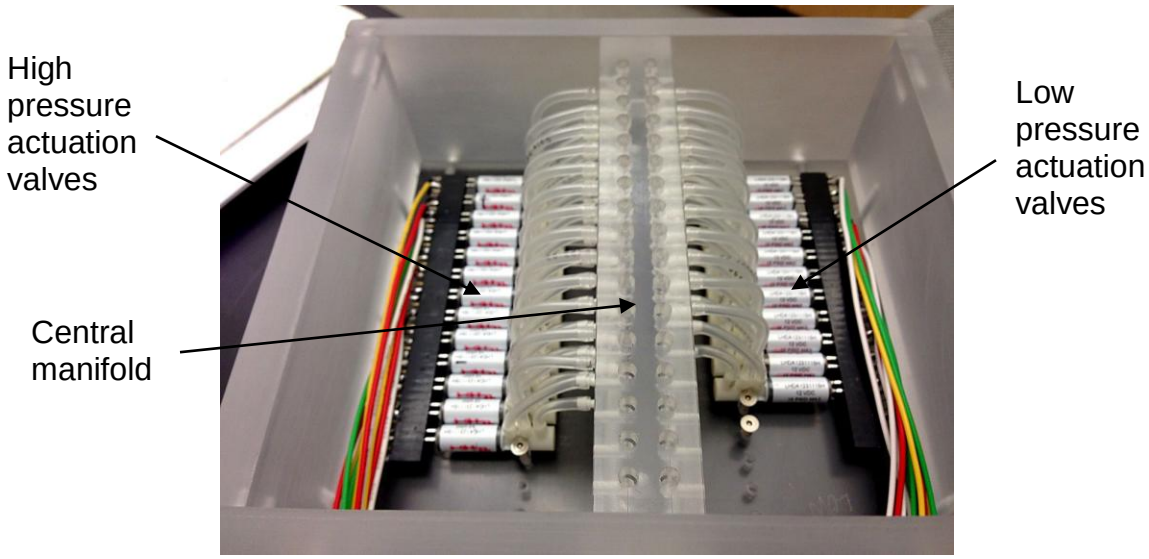

Figure 20: Central manifold inside Minson's pneumatic machine with high pressure actuators connected on the left and low pressure ones on the right.

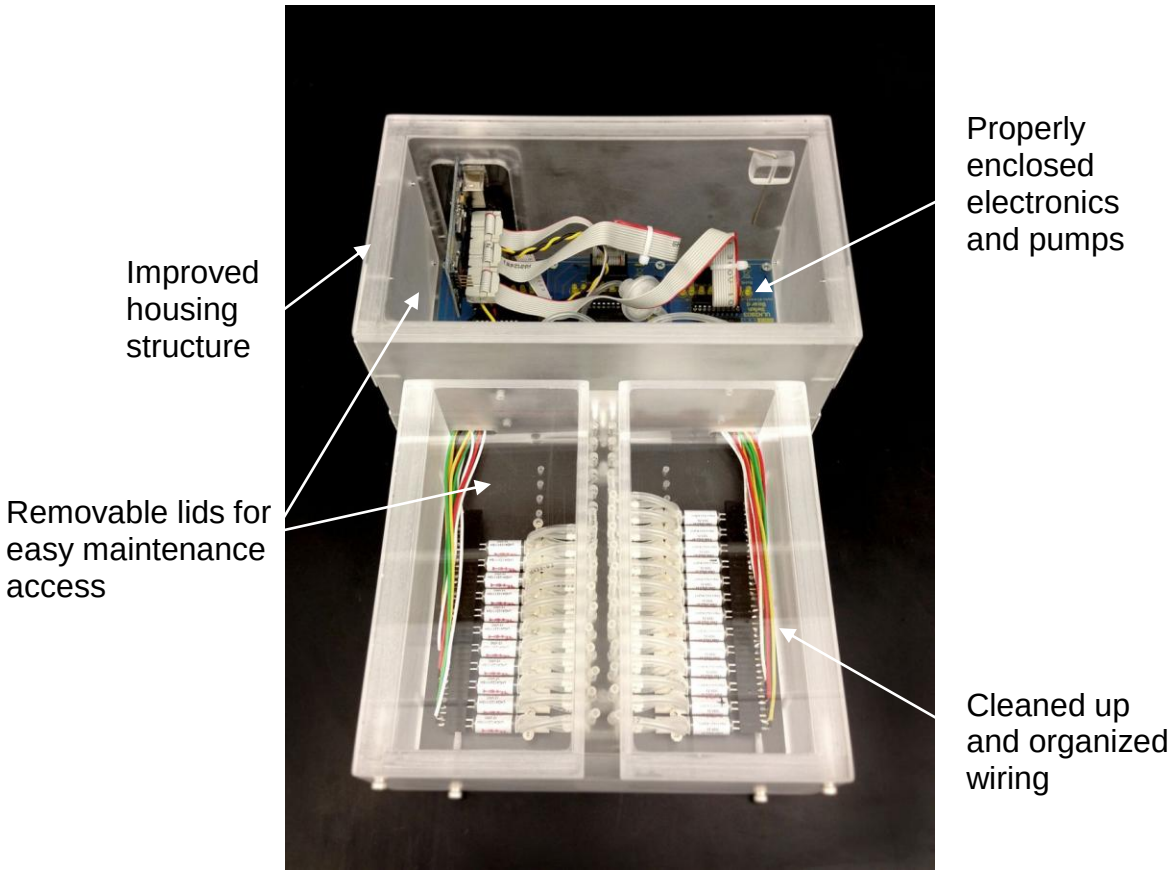

Figure 21: Minson's newly constructed pneumatic machine with design improvements.

CHAPTER 3

RESULTS AND DISCUSSION

As the new materials and design concepts were conceived and implemented, various tests were performed along to way to determine design validity. The functionality and reliability of the remodeled pneumatic machine will be discussed in detail and Johnson's PDMS chip and the new PC chip will be compared for manufacturing time, material cost and NA extraction results.

The First Prototype

To prove the new concept of replacing the bottom portion of the chip with the manifold block, a small scale prototype was constructed. Two pieces of 6mm acrylic were used as the support structure, sandwiching the PC chip. The manifold block (bottom support structure) had a reservoir pocket machined 3mm deep and a thin .5mm raised plastic gasket glued around the pocket and the five valves. Each of these features had holes drilled through the bottom to connect to the pneumatic actuation machine. The prototype with pneumatic connections is shown in Figure 22.

The lid was a plain flat piece of acrylic with tapped holes in the corners allowing it to be screwed tight from the back side. The components were

clamped together with four #6-32 screws and connected to Minson's pneumatic machine for a verification test. A small laminated PC chip was designed with five valves branching out from a reservoir-pump. Five small wells were fastened to the chip via double-sided tape to hold liquids for testing at the inlets. A photograph of the chip is shown in Figure 23.

For the experiment, one well was filled with water while the others remained empty. The reservoir-pump was vacuum pressurized (open position) and the valve to the water was opened. The water emptied from the exterior well into the reservoir-pump. The first valve was closed and a different one opened. The reservoir-pump was then pressurized (closed position), forcing the water out and into another exterior well. This process was repeated for each of the valves and wells until they had all been tested. The demonstration successfully proved the design concept was sound and the chip capable of working with the pneumatic machine pressures (-34kPa to 69kPa).

Second Prototype

After the first successful proof of concept prototype was demonstrated and other features designed for the chip, a rough, full-scale prototype was prepared for a complete proof-of-concept test. This intermediate step was taken before a full and final redesign to make sure nothing critical was overlooked. The second prototype also served as an opportunity to learn more about the new system and to assist in the final details of the design.

The bottom manifold block was manually machined on a Bridgeport mill using an acrylic plate. To avoid material flexure and ensure a flat sealing surface, 19mm acrylic plates were used for both the clamping lid and manifold block. Hoses were glued to the bottom of the manifold block for pneumatic actuation. C-clamps provided the sealing force needed for operation and the new prototype was attached to the pneumatic machine and control system. The full-sized chip successfully pumped water in from a sample, mixed between reservoir-pumps, flowed through the filter and pumped back out to waste.

Minson's Pneumatic Machine and PC Chip Results

Minson's pneumatic machine is organized, user friendly and easy to work with. The redesigned central manifold pressure distribution system eliminated all leaks and created a far more reliable system, which became clear as no additional time was spent fixing or troubleshooting Minson's pneumatic machine. The new PC chip successfully performs the necessary functions associated with sample preparation and NA extraction including fluid connection, valving, pumping, mixing, and filtering. The PC chip has 16 fluid inlet/outlet connections, 8 reservoir-pumps and 33 valves. These features allow a large range of sample preparation protocols, NA extractions and other microfluidic applications.

Manufacturing Johnson's PDMS chip is a time consuming and labor intensive task. The PDMS is mixed, degassed, poured in a mold, cured, removed and bonded. To achieve proper bonding, each individual reservoir and valve is masked with a proper sized piece of tape for air plasma bonding. The pieces are

then removed and the chip aligned for adhesion. Not only are these tasks tedious and lengthy, but they require highly skilled personnel. The time to manufacture one of Johnson's PDMS chips averages about 4 hours.

In contrast, the new PC chips are much simpler to make and require much less time. The PC chip is cut out with a laser. Loose debris is removed from the layers. A glass microfilter is inserted between the layers and the PC chip is placed in a heat press for thermal bonding. Once removed, the PC chip is air plasma treated along with the silicone rubber layer and returned to the heat press for 30 more seconds. The average time to make a PC chip is about 15 minutes. Thus, the new PC material and manufacturing methods are 16 times faster than Johnson's PDMS chip.

For a comparison of the cost difference between the PDMS and PC chips, only the bulk material will be considered. Other factors like assembly time, skilled personnel, machinery, and so forth also play a significant role, but remain more subjective so they are excluded from the cost analysis. The PDMS is purchased in a 18,927cm^3 volume for about $1,500. One cubic cm of PDMS is $0.08 and each PDMS chip has a volume of about 164 cm^3, bringing the cost of each chip to around $13.00. The PC is purchased in 14m^2 rolls for $140. One square cm of PC is about 0.1 cents. There are approximately 237 square cm of PC per chip, bringing the cost to around $0.24 each. Thus, the cost of materials for the new PC chips are nearly 1/60th the cost of the PDMS chips.

Extraction Testing

The new PC chips and Minson's pneumatic machine was tested to verify biological compatibility, time performance and extraction efficiency. Extraction procedures were performed utilizing human genomic DNA at a concentration of 54ng/µl. The sample was used in conjunction with QIAamp DNA blood mini kit (Qiagen) reagents and protocol.

Before beginning the extraction, a 200µl sample of blank (no DNA) AE Buffer was passed through the chip channels, reservoir-pumps, filter and collected. The sample was loaded via reservoir-pump 15 and passed through reservoir-pumps 16, 22, 23 and out to waste. A diagram of the reservoir-pump layout is shown in Figure 24.

Next, a mixture of 20µl DNA with 200µl AE Buffer followed the same procedure. Flushing these fluids through the chip was done to verify the materials used in the chip, filter and flexible membrane had minimal effect on the fluorescence results when tested in the plate reader. 20µl of purified DNA were mixed with 200µl of AL Buffer and 200µl of 100% ethanol. This mixture was pumped into the chip through reservoir-pump 16 and mixed 5 times via shuttling between reservoir-pumps16 and 22. The fluid was then passed through the glass microfilter into reservoir-pump 23 and then exited out to waste. 500µl of Buffer AW1 entered reservoir-pump 16 and passed through the filter to reservoir-pump 23 and out to waste. 500µl of Buffer AW2 followed the same procedure. The filter was then dried using forced air from Minson's pneumatic machine for 10 minutes. As a final step, the filter was eluted with 100µl of AE Buffer. The buffer entered

the chip through reservoir-pump 19, passed through the filter, into reservoir-pump 20 and out to collection. The extraction test was performed in 23 minutes and was fully automated.

Minson's pneumatic machine is controlled with a LabView software program created by Johnson. The graphical user interface is shown in Figure 25. The software can be programmed to automatically control valve and pump actuation or it can be done manually using on-screen buttons. Multiple extraction tests can be easily programmed to run automatically. Single procedures can be performed quickly using the manual mode. For this extraction test, the automated mode was utilized.

The PicoGreen Assay Kit (Invitrogen) was used with a fluorescence detection microplate reader (Biotek) to quantify the results of the extraction test. The microplate was loaded with 100µl samples of five fluids. These fluids included: AE Buffer, AE Buffer passed through the chip, a purified DNA sample, a DNA sample run through the chip (without extraction) and the DNA extracted from the chip. Each of these samples was mixed with 100µl of PicoGreen fluorescence diluted 200:1 with DI water. The microplate reader produced the results shown in Figure 26.

The DNA sample fluoresced at approximately 23,000 Fluorescence Units (F.U.s). The DNA run through the chip read only slightly less, indicating a small amount of DNA may have been deposited or lost on the chip/filter, or the reduction could be attributed to noise within the plate reader's results. A similar result can be seen for AE Buffer. The buffer that passed through the chip had a

slightly higher reading than the AE Buffer that was not in contact with the PC chip. This may indicate the fluid picked up some fluorescence contaminants, but the detection difference is likely the result of noise. The DNA collected through the extraction procedure was about 12% of the total DNA. While this result is less than the desired 30% efficiency, the extraction procedure performed was not an optimized process. The protocol and reagents utilized in the extraction procedure were taken from a commercial whole blood DNA extraction spin kit. This commercial extraction kit was not intended for purified human genomic DNA extractions. A better approach would include specific protocol and reagents for the sample being extracted. It would also be beneficial for the extraction to be performed by an experienced individual in an associated field of study. It is likely that these changes would significantly improve the extraction efficiency of the new PC chip.

Iterating through quick and multiple prototypes helped validate the effectiveness of new concepts and designs. This approach assisted in the successful design of a versatile and disposable PC extraction chip. The new PC chip effectively reduced material cost and manufacturing time while successfully extracting DNA. The extraction efficiency was less than the imposed constraint due to nonoptimized protocols, but the PC chip and pneumatic machine performed the procedure within the 30 minute timeframe. Further work is suggested to optimize DNA extraction efficiency in the future and is beyond the scope of this thesis.

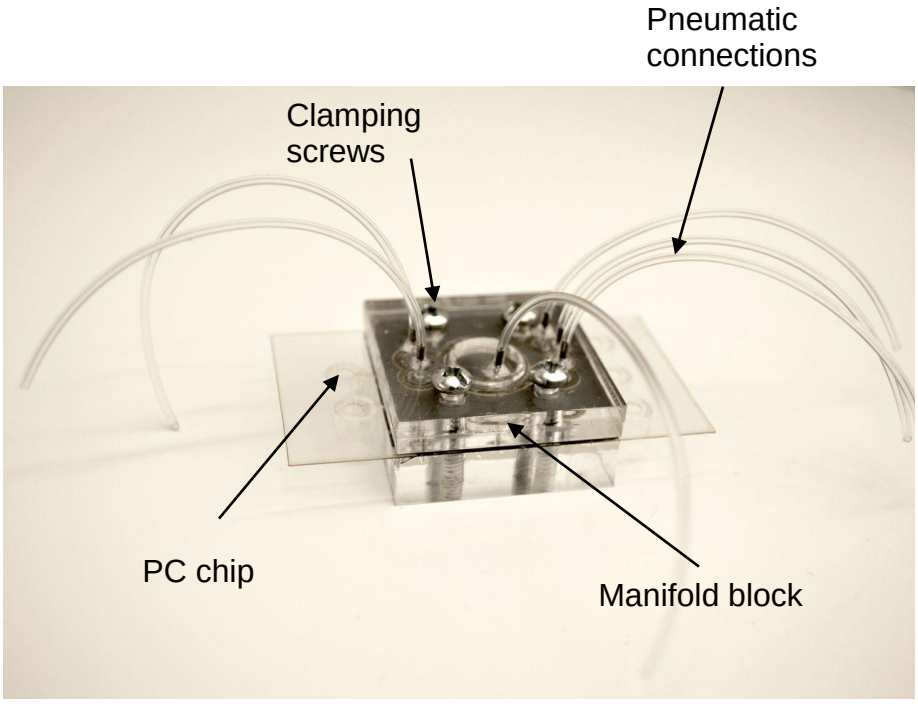

Figure 22: First valve and reservoir prototype showing bottom side with clamping screws and fluid connections

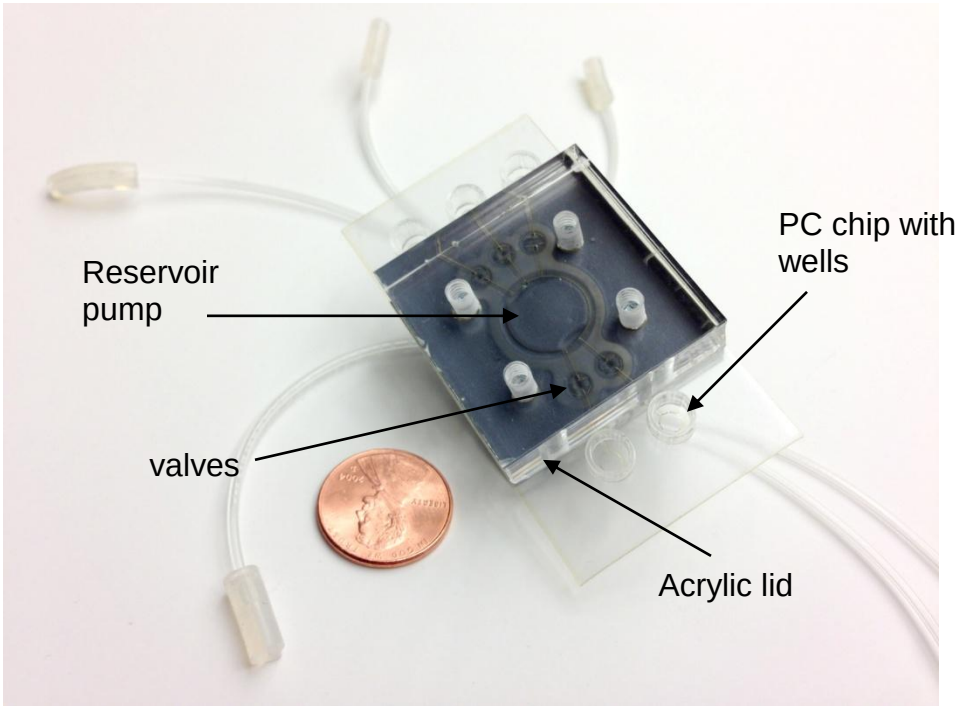

Figure 23: First proof of concept test with a liquid sample; five valves and a reservoir pump were tested with Minson's pneumatic machine.

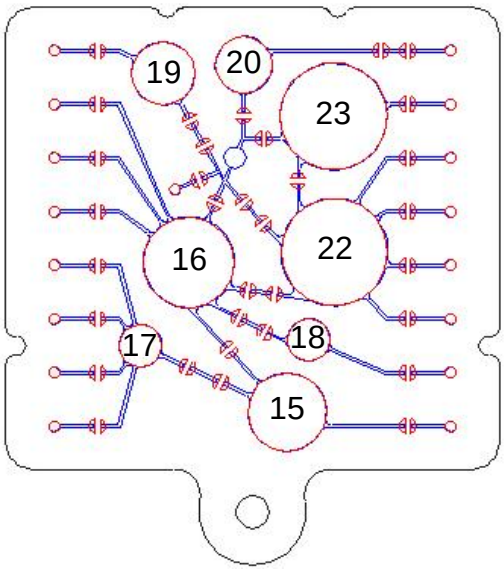

Figure 24: Layout of the reservoir-pump number schematic.

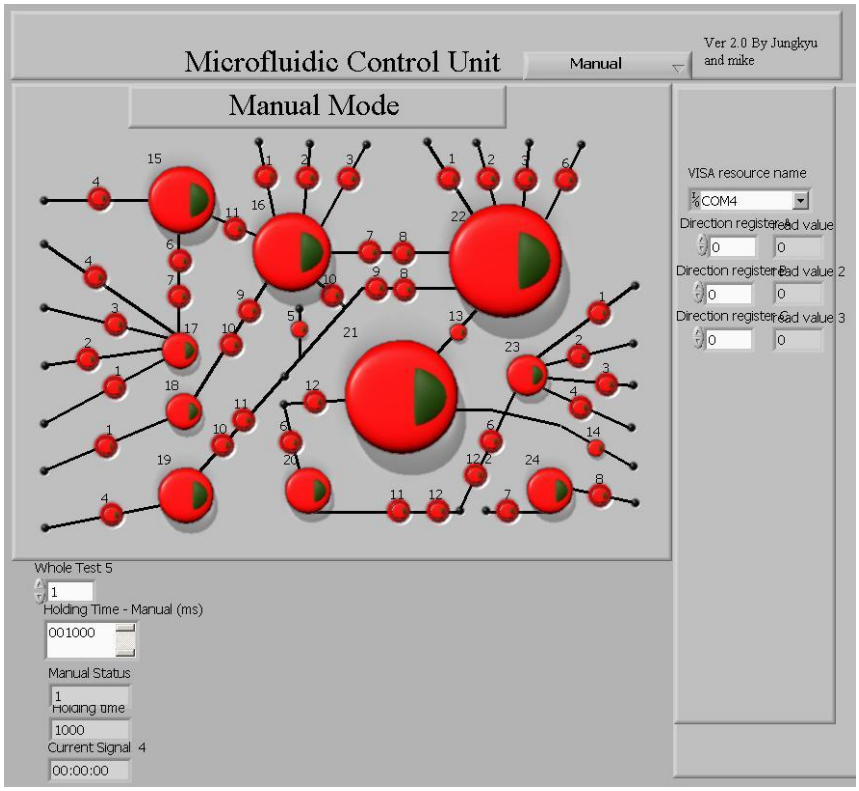

Figure 25: LabView software control program for the pneumatic system and microfluidic chip.

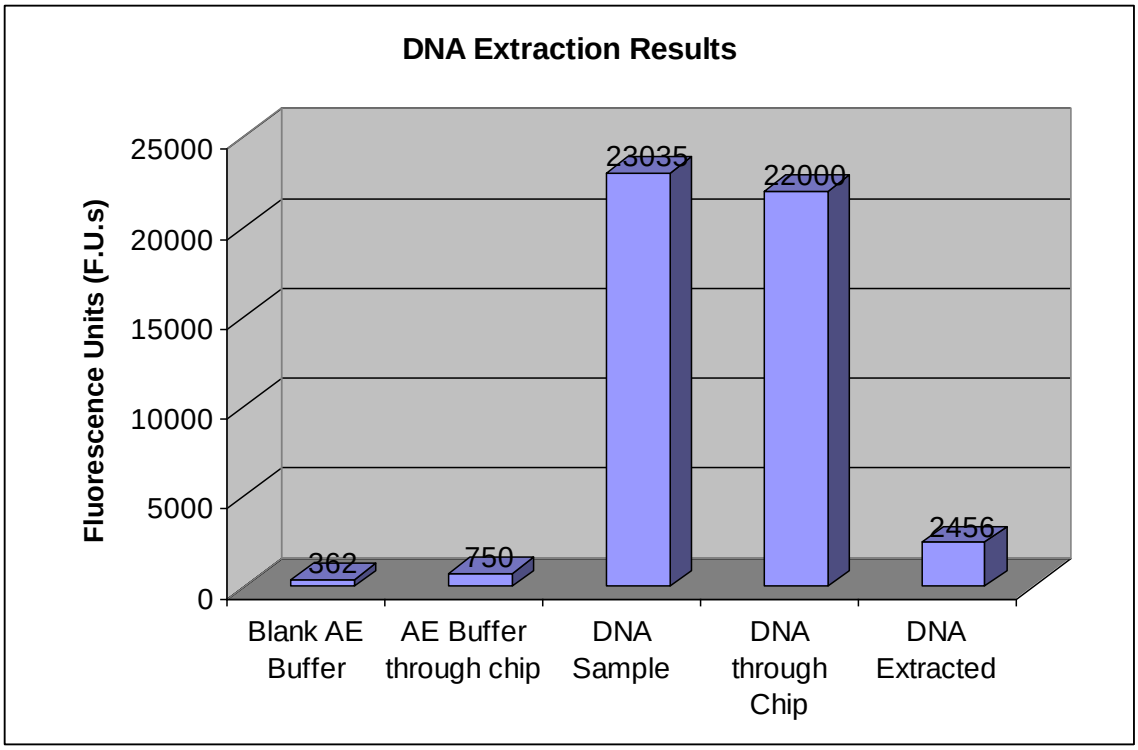

Figure 26: Graph showing results of DNA extraction performed with PC chip.

CHAPTER 4

CONCLUSION

Microfluidic systems have displayed a number of advantages over traditional methods for extracting NAs, but few are versatile enough to handle a wide range of biological sample preparation processes. In this work, a lower-cost, versatile, disposable microfluidic chip has been developed with supporting machinery to extract NA's and perform necessary sample preparation steps for a wide variety of raw biological samples.

This new system was developed on the shoulders of Johnson's PDMS chip. While Johnson’s chip performed NA extraction and sample preparation well, it was relatively expensive and slow to manufacture. The specific objectives of this work were to improve the Johnson design by reducing material costs by a factor of 10 and decreasing manufacturing time by half. Reducing the microfluidic chip cost enabled the newly designed chip to be disposable, eliminating washing cycles between tests and the potential for cross contamination. In addition to cost reduction, the constraints for the project included using materials that were biologically compatible, designing the chip to function with Johnson’s pneumatic machine, perform NA extraction in a 30 minute time frame and extract at least 30% DNA from a known concentration. To satisfy these objectives and

constraints, a novel, composite chip made from laminated layers of PC and a silicone rubber membrane was developed.

The most significant contribution of this work was the introduction of the manifold block to the pneumatic machine. By removing the lower portion of Johnson's PDMS chip and making it a permanent part of the pneumatic machine, the new PC chip complexity and cost was significantly reduced. This modification allowed the PC chip to remain planar and simple while including all the necessary features such as flexible membrane valves, reservoir-pumps, fluid inlet connections and a glass microfilter for solid phase extraction.

In addition to developing the new, disposable PC chip, Johnson's pneumatic machine was redesigned and rebuilt using a central manifold to reduce the number of hoses and connections, thereby eliminating problematic leaks. Minson's improved pneumatic machine was designed for easy maintenance access and properly housed electronic and pneumatic components. Minson's pneumatic machine also included access ports for other prototypes or postprocessing procedures such as PCR.

As a result of material and design improvements, a versatile and disposable microfluidic system was developed and capable of executing a variety of biological sample preparation procedures and NA extractions. The new PC chip is produced in 1/16th of the time (15 minutes) and at 1/60th of the cost ($0.24) of Johnson's PDMS chip. Thus the objectives to reduce cost by 10 times and manufacturing time by half were more than satisfied. The new PC chip was also biologically compatible, as demonstrated with the successful extraction of

DNA. The new PC chip also functioned with the existing pneumatic machine and successfully performed NA extraction within the 30 minute time frame. Thus nearly all design constraints were satisfied. Initial DNA extraction testing with the PC chip reached efficiencies of only 12%. Although this failed to meet the 30% efficiency constraint, substantial optimization of protocols remains incomplete. It is expected that extraction efficiencies could be improved by individuals with NA extraction experience.

Future Work

While great strides were made toward a more cost effective and versatile extraction system, the general approach of this work was a broad overview. As a result, a number of features would benefit from further analysis. Detailed analysis could be performed on filter size and characterization, performance of the flexible membrane material, characterization of chip shrinkage and optimization of extraction efficiencies. In addition to more detailed analysis, the pneumatic machine could benefit from supplementary features like postextraction amplification and detection.

A final feature that would greatly increase system versatility would be adjustable volume control for the reservoir-pumps. This could easily be accomplished by incorporating a screw-adjustable bottom in the reservoir-pump wells. This would allow the pneumatic machine to be designed with a simpler, more uniform layout, eliminating excess valves. It would also allow the user

greater control for sample preparation protocols that are sensitive to volumetric ratios.

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